International Journal of Agricultural Science and Research (IJASR)
ISSN(P): 2250-0057; ISSN(E): 2321-0087
Vol. 8, Issue 3 Jun 2018, 67-72

A SIMPLE MODIFIED DNA EXTRACTION METHOD OF MAIZE

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ABSTRACT

Marker Assisted Selection (MAS) is becoming an efficient method of crop improvement programme for maize and other crops. For this method, it requires quality genomic DNA extraction of the large sample. The present study described a simple, cost-effective miniprep DNA extraction method without using liquid nitrogen from the leaf of maize that can be performed in a laboratory with basic facilities. Using this method genomic DNA was extracted from lines derived from CAU-87. The ratio of absorbance at 260 nm to 280 nm ranges from 1.833 to 2.2 and the concentration of DNA (ng/µL) vary from 60ng/µL to105 ng/µL. The PCR amplification using three pairs of crtRB1 3'TEgene-specific primers yield an acceptable result.

KEYWORDS: Maize, DNA Extraction & Crtrb1 3' TE Gene-Specific Primer

Received: Mar 29, 2018; **Accepted:** Apr 19, 2018; **Published:** May 03, 2018; **Paper Id.:** IJASRJUN20186

Original Article

INTRODUCTION

Maize is one of the three important cereal crops after wheat and rice consumed as a staple food (Mukherjee, 1989). It is known as the queen of cereals, globally because of its highest genetic yield potential among the cereals and cultivated on nearly 150 mha in about 160 countries having a wider diversity of soil, climate, biodiversity and management practices that contribute 36 % (782 mt) in the global grain production. (Nand *et al.*, 2018). Recently, many specific target genetic studies have been done in this crop *viz.* flowering time (Thornsberry *et al.*, 2001; Pressoir *et al.*, 2009), kernel composition (Wilson *et al.*, 2004), carotenoid contents (Harjes *et al.*, 2008; Yan *et al.*, 2010, Zhang *et al.*, 2012, Babu *et al.*, 2013; Muthusamy *et al.*, 2014). Marker Assisted Selection (MAS) is becoming an efficient method for crop improvement programme due its precise and accelerated approach; development of PCR based markers such as simple sequence repeat (SSR or microsatellites) has also made it one of the preferable method because of its high level of polymorphism, simplicity, requirement of DNA in low quantity and low cost. Eathington *et al.* (2007) showed that MAS methodologies increased the mean performance of progeny as compared to the conventional breeding methodologies.

Genomic DNA extraction is the first steps for studying genetic aspects of an organism. Quality DNA extraction from cereals is generally difficult due to the presence of polysaccharides, proteins, and DNA polymerase inhibitors and it affects the quality and quantity of the isolated DNA, rendering the sample non-amplifiable (Sarwat *et al.*, 2006). Many DNA extraction protocols have been developed in plants (Doyle and Doyle, 1987; Saghai-Maroof *et al.*, 1984; Dellaporta *et al.*, 1983), however, it requires long time-consuming steps, laborious works and liquid nitrogen which is hard to procure. On the other hand, the mechanical grinding of the sample for maize in extraction buffer is more cost-effective than using liquid nitrogen (Abdel-Latif and Osman, 2017). Many commercial DNA extraction and purification kits are available nowadays, but it is expensive and not a good option for MAS due to its requirement in large scale. The aim of our study is to develop a rapid and reliable genomic DNA extraction protocol suitable for MAS in maize and in a

laboratory with basic facilities.

MATERIALS AND METHODS

Materials

Lines derived from CAU-87 were used for the study. Seeds were germinated in an incubator and planted in the laboratory.

DNA Extraction

Genomic DNA was extracted using the method described Zheng *et al.* (1995) with some modifications; leaves of 15 days old maize plantlets were cut into approx 0.5 cm^2 to 1 cm^2 using paper puncher (subsequent cleaning is required after each sample), then were surface sterilized using 70% ethanol. The leaflets were collected in a 1.5ml microcentrifuge tube and 100 µL of extraction buffer (50 mM Tris-HCl, 25 mM EDTA, 1% SDS, 300 mM NaCl) were added. The leaflets were then grind using a micro pestle (Tarson, India). Then another 300 µL of extraction buffer was added to the micro centrifuge tube. The mixture was kept in a water bath for 10 mins at 65° C. Then an equal volume of chloroform: isoamyl alcohol (24:1) and tris-saturated phenol was added to the mixture (200 µL of chloroform: isoamyl alcohol and 200 µL of Tris-saturated phenol). After centrifuging for 1 min at 15000 RPM, the supernatant was collected in a new microcentrifuge tube. Then 800 µL of ice-cold isopropanol was added and mix by inverting the tube. Then the mixture was again centrifuged for 5 mins at 15000 RPM and the supernatant was discarded. The pellets were washed with 70% alcohol and dried. Then the pellets were dissolved in 50 µL TE buffer and stored at -20° C.

Assay of DNA Yield and Purity

Absorbances (*A*) at 260 nm and 280 nm were measured using CE7200 7000 series UV spectrophotometer (Cecil, UK) for determining the yield and purity of the DNA.

PCR Analysis

To determine the reliability and applicability of this method, three pairs of crtRB1 3'TEgene-specific primers, crtRB1-3'TE-F: ACACCACATGGACAAGTTCG, crtRB1-3'TE-R1: ACACTCTGGCCCATGAACAC and crtRB1-3'TE-R2: ACAGCAATACAGGGGACCAG (Yan *et al.* 2010; Vignesh *et al.*, 2012; Selvi *et al.*, 2014) were selected for PCR amplification. The primers were synthesized and purified by IDT (Integrated DNA Technologies). PCR was performed with a total volume of 25 µL, containing 1 µL template DNA, 2.5 µL of PCR buffer(supplied with Taq polymerase), 0.2 mM each of dNTPs, 0.75 µM of each primer and 2 U of *Taq* DNA Polymerase (GeNei, Bengaluru, India). PCR amplification (2720 Thermal Cycler, Applied Bio systems, California, USA) was carried out using the standard cycle conditions as given by Selvi *et al*., 2014. The PCR products were separated by electrophoresis on 2% agarose gel. The gels were stained with ethidium bromide and detected under Gel Doc XR+ with Image Lab Software (Bio-Rad, California, USA).

RESULTS AND DISCUSSIONS

DNA Yield and Purity

The ratio of absorbance at 260 nm to 280 nm ranges from 1.833 to 2.2 and the concentration of DNA (ng/µL) varies from 60 ng/µL to 105 ng/µL (Table 1). The purity of DNA was slightly low due to RNA contamination, but it doesn't affect the PCR amplification (figure 2).

Table 1: Quantitative Estimates of DNA Concentration Revealed by Spectrophotometer

Sl. No	Maize Line	A_{260}	A_{280}	A_{260}/A_{280} Ratio	DNA Concentration (Ng/Ml)
1.	CAU-87-1	0.024	0.013	1.846	60
2.	CAU-87-2	0.025	0.012	2.083	62.5
3.	CAU-87-3	0.029	0.016	1.813	72.5
4.	CAU-87-4	0.029	0.014	2.071	72.5
5.	CAU-87-5	0.023	0.011	2.090	57.5
6.	CAU-87-6	0.042	0.022	1.909	105
7.	CAU-87-7	0.028	0.013	2.154	70
8.	CAU-87-8	0.034	0.016	2.125	85
9.	CAU-87-9	0.022	0.010	2.200	55
10.	CAU-87-10	0.033	0.017	1.941	82.5
11.	CAU-87-11	0.039	0.021	1.857	97.5
12.	CAU-87-12	0.026	0.012	2.166	65

PCR Amplification

The genomic DNA extracted without using tris-saturated phenol amplified only 296 bp, (Lane 2) with crtRB1-3'TE primer and with Tris-saturated phenol amplified all the three bands (296 bp, 543 bp and 1221 bp) in CAU-87-8 line. Further, the DNA sample was diluted to 10 times and 100 times using molecular grade water. PCR product is observable with no dilution to 10 times dilution but 100 times dilution was not amplified properly showing faint and missing band (Lane- 5, 6 and Lane-7, 8; Figure 1) indicating that the DNA template extracted by our method can be diluted up to 10 times which further increase the quantity of DNA template. PCR amplification using crtRB1-3'TE markers were all acceptable for all the maize line as shown in figure 2.

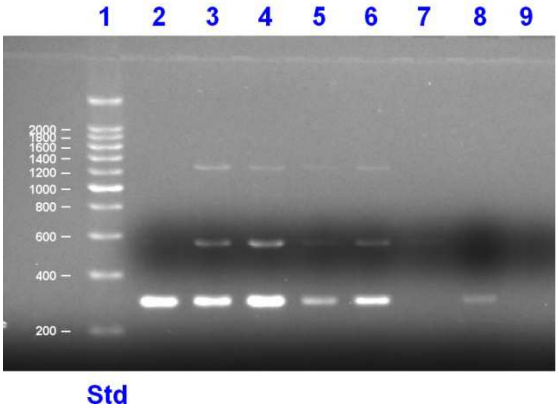

Figure 1: PCR Amplification of CAU-87-8 With Crtrb1 3'TE Gene Specific Primer.
Lane 1- 200bp DNA Ladder; Lane 2- Extraction Without Phenol;
Lane 3, 4- Extraction With Phenol Without Dilution;
Lane 5, 6- Extraction With Phenol and 10 Times Dilution,
Lane 7, 8- Extraction With Phenol and 100 Times Dilution;
Lane 9- Blank

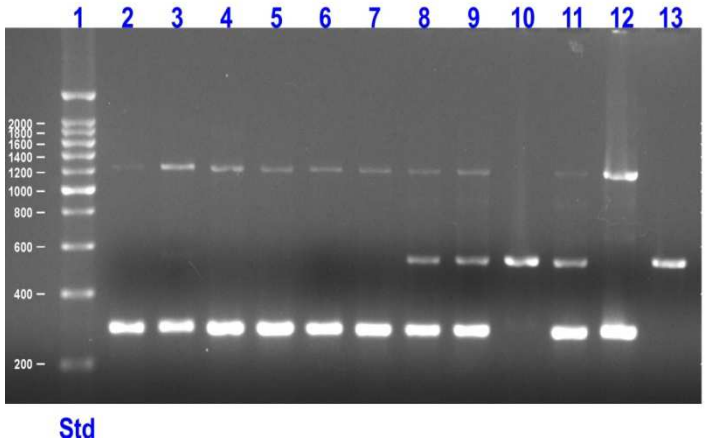

Figure 2: PCR Amplification of 12 Maize Lines with 3 Pairs of Crtrb1 3'TE Gene Specific primer. Lane 1- 200bp DNA Ladder; Lane 2 to Lane 13- Twelve Maize Lines

CONCLUSIONS

The present modified method of DNA extraction of Maize is suitable for PCR analyses, especially in foreground selection in Marker Assisted Selection where it requires a lot of lines to be handled. Further, the method is cheap and can be performed within a short period of time in a laboratory with basic facilities.

ACKNOWLEDGEMENTS

The authors are much obliged to College of Agriculture, CAU, Imphal for providing research materials and support for the research works.

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